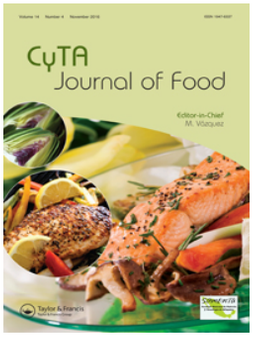

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# Modification of glucosinolates in turnip greens (*Brassica rapa* subsp. *rapa* L.) subjected to culinary heat processes

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**ABSTRACT**

The consumption of *Brassica* vegetables has been related to improved health benefits due to their phytochemical components, such as glucosinolates, that induce a variety of physiological functions. Glucosinolate levels can be affected when they are submitted to heat treatments before consumption. This paper investigates, by an HPLC-DAD method, the effect of three cooking treatments on the nutritional quality of turnip greens. Fresh turnip leaves were homogenized with sand and Milli-Q water and centrifuged. They remained there for 3 h for natural autolysis. Samples, fresh and processed, were extracted with dichloromethane; the organic extract had been evaporated to dryness, was dissolved in acetonitrile and injected into the chromatograph. Eight compounds were identified: allyl-isothiocyanate, erucin, sulforaphane, iberin, napin, goitrin, benzyl- isothiocyanate and phenethyl-isothiocyanate. Napin was the major compound in all samples followed by goitrin. The best treatment is steam cooking, followed by pressure-cooking, while the boiling treatment produced a 60% loss.

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**KEYWORDS**
Glucosinolates; myrosinase; turnip greens (*Brassica rapa* subsp. *rapa* L.); HPLC-DAD; culinary treatments

**PALABRAS CLAVE**
Glucosinolatos; mirosinasa; grelos (*Brassica rapa* subsp. *rapa* L.); HPLC-DAD; tratamientos térmicos

## Modificación de glucosinolatos en grelos (*Brassica rapa* subsp. *rapa* L.) sometidos a procesos térmicos culinarios

**RESUMEN**

El consumo de vegetales *Brassica* se relaciona con buena salud debido a su contenido en glucosinolatos, componentes fitoquímicos, responsables de una variedad de funciones fisiológicas. Los niveles de glucosinolatos en las *Brassica* pueden ser afectados cuando se someten a tratamientos por calor, previamente a su consumo. El objetivo de este trabajo es investigar, mediante un método de HPLC-DAD, los efectos de tratamientos culinarios en la calidad nutricional de los grelos. Las hojas de grelo frescas son homogeneizadas con arena y agua Milli-Q y centrifugadas, dejándolas 3 h (37 °C) para su autolisis natural. Las muestras, frescas y procesadas, se extraen con diclorometano; el extracto orgánio se evapora a sequedad, se disuelve en acetonitrilo y se inyecta en el cromatógrafo. Se han identificado ocho compuestos: alil-isotiocianato, erucina, sulforafano, iberina, napina, goitrina, bencil-isotiocianato and fenetil-isotiocianato. La napina es el mayoritario en todas las muestras seguido de goitrina. El tratamiento que produce menos pérdidas es la cocción a vapor, seguido por la cocción bajo presión, mientras que el hervido da lugar a una pérdida del 60% del contenido.



## 1. Introduction

The potential benefits of fruit and vegetable consumption, in the reduction of cancer in human health, have been long recognized (Herr & Büchler, 2010; Mahn & Reyes, 2012). Vegetables belonging to the Brassicaceae family are widely consumed throughout the world and have attracted great interest as a source of phytochemical components in human nutrition (Cartea & Velasco 2008; Akhlaghi & Bandy 2010).

Glucosinolates (GLS) all share a chemical structure consisting of *β*-D-glucopyranose residue linked via sulphur atom to a (Z) -N-hydroximinosulfate ester with a variable side chain derived from amino acids. GLS can be grouped into three chemical classes, aliphatic, aromatic and indole. There are over 100 known types of glucosinolates, about 30 of them were found in *Brassicas*. These compounds are a defense against pests and diseases. Although intact glucosinolates may provide resistance to herbivorous insects, fungi, and microorganisms (Al-Gendy, El-Gindi, Hafez, & Ateya, 2010; Matthew, Hall, Jobling, & Gordon, 2015); the defensive properties are increased when tissues are fragmented by mechanical damage, infection, etc.

The glucosinolates present in vacuoles are hydrolysed by the enzyme myrosinase (β-thioglucosidase glucohydrolase) that undergoes the Lossen rearrangement to produce isothiocyanates. Isothiocyanates display diverse and interesting biological properties and can be hepatotoxic, goitrogenic and/or anti-carcinogenic; they have cancer-preventive potential, primarily as inducers enzymes of Phase I and II, representing a complex system response to changes in the level of oxidation at the cellular level, a master control system which induces activation of protective genes from cells (Herr & Büchler, 2010, Mahan & Reyes, 2012).

Phytochemicals are found in all plant parts; the seeds, roots, and the inflorescences are the parts that have higher concentrations of glucosinolates, followed by the leaves and finally the stems (Campas-Baypoli, Sánchez-Machado,

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Bueno-Solano, Ramírez-Wong, & López-Cervantes, 2009). Other factors that may potentially influence and alter the content of glucosinolates are cultural practices, storage conditions, and preparation of food (Yábar, Pedreschi, Chirinos, & Campos, 2011).

Most vegetables are subjected, for consumption, to heat treatment at home or in industry. It is known that cooking induces significant changes in chemical composition, influencing the concentration and bioavailability of bioactive compounds (Gao-Feng, Bo, Jing, & Qiao-mei, 2009; Francisco, Velasco, Moreno, García-Viguera, & Cartea, 2010; Jones, Frisina, Winkler, & Tomkins, 2010; Clariana, Valverde, Wijngaard, Mullen, & Marcos, 2011; Tanongkankit, Chiewchan, & Devahastin, 2011; Hanschen, Bauer, Mewis, Keil, Schreiner, Rohn, & Kroh, 2012; Korus, Słupski, Gebczynski, & Banas, 2014; Xu, Zheng, Yang, Cao, Shao, & Wang, 2014). Glucosinolates and their hydrolysis products are primarily lost from *Brassica* vegetables by leaking into the cooking water, but the rate and extent of loss depend on the type of treatment used, the cooking time and/or the amount of water used (Song & Thornalley, 2007).

There is thus a need for more studies to investigate the effects on several groups of phytochemical compounds with potential benefit to human health, when they are subjected to thermal treatment. In this work, the modification of glucosinolates when the turnip was subjected to cooking processes (pressure, steam and boiling treatments) has been evaluated by the HPLC-DAD method. There is high consumption of turnip greens in the traditional diet of Galicia.

## 2. Experimental

### 2.1 Standards and reagents

Ascorbic acid, enzyme myrosinase (thioglucosidase from *Sinapis alba*) (white mustard) seed], glucosinolates: glucoerucin (4-methylthiobutyl-glucosinolate), glucoiberin (3-methylsulfinylpropyl-glucosinolate), gluconapin (3-butenyl-glucosinolate), glucoraphanin (4-methylsulfinylbutyl-glucosinolate), progoitrin (2(S)-hydroxy-3-butenyl-glucosinolate), sinigrin (allyl-glucosinolate), and isothiocyanates: benzyl-isothiocyanate (benzylITC), ethyl-isothiocyanate (ethylITC), isopropyl-isothiocyanate (isopropylITC) and phenethyl-isothiocyanate (phenethylITC), were from Sigma-Aldrich (Steinheim, Germany). Analytical grade acetonitrile (ACN) and dichloromethane (DCM) were purchased from Merck (Darmstadt, Germany). Washed sea sand was from Panreac (Barcelona, Spain).Water was obtained from a Milli-Q water purification system (Millipore) (Bedford, MA, USA).

Stock solutions of 1000 mg/l in Milli-Q water:ACN (95:5) of each of the standard solutions of glucosinolates and isothiocyanates were prepared. Working solutions of individual compounds and their mixtures were prepared from stock solutions by dilution in Milli-Q water.

### 2.2 Hydrolysis from glucosinolates

0.2 ml of each stock solution of 1000 mg/l was placed in a centrifuge tube of 13 ml. 2.5 (unit sigma) myrosinase enzyme and 2 mg ascorbic acid and Milli-Q water up to 5 ml were added. Hydrolysis takes place for 3 hours at 37 ºC in an oven. Then 5 ml of DCM is added, shaken in vortex for 5 min (IKA Vortex Genius3 IKA-Werke Gmbh&Co. KG, Germany) and centrifuged for 10 minutes at 3500 rpm.

The obtained organic layer eluate was driedby a nitrogen stream at 0 ºC. The residue was reconstituted in 1 ml ACN and the solution was filtered through about a 0.50 µm syringe filter of PTFE (Advantec, Toyo Roshi Kaisha, Ltd., Utsunomiya-shi, Japan).

The method was applied to four samples of turnip greens (*Brassica rapa* subsp. *rapa* L.) purchased in the market of Santiago de Compostela (Galicia, northwest Spain).

Prior to analysis, samples were washed, sliced, and chopped. The moisture content was determined in samples from the weight loss by drying 5 g of the sample above constant weight, in a conventional oven.

1 g of fresh turnip leaves (raw control), was crushed in a mortar with 0.1 g of sand and Milli-Q water. It is poured into a 13 ml centrifuge tube and increased to 9 ml with Milli-Q water. It remained for 3 hours at 37 °C, for natural hydrolysis. Samples were extracted three times with 3 ml of DCM, shaken in vortex for 10 min and centrifuged at 3500 rpm for 10 min. The organic layer eluate was evaporated to dryness under a nitrogen stream at 0 ºC, redissolved in 1ml ACN, filtered through about a 0.50 µm syringe filter of PTFE and was analyzed by HPLC. The samples were analyzed by duplicates.

The samples (20 g of turnip greens) were subjected to three common cooking methods. Steaming was carried out using a steam insert with the 20 g of turnip greens suspended above 100 ml of boiling water for 10 min covered by a lid. Boiling. vegetable material (20g) was added to boiling tap water in a covered stainless-steel pot (1:5 food/water) and cooked for 15min. For high-pressure-cooking, the leaves (20g) were immersed in 100 mL of cold water and cooked for 7 min under high-pressure in a pressure cooker. After treatment the turnip greens were drained and were subjected to the same extracted procedure for fresh vegetable. The samples were analyzed by duplicates.

Analyses performed by HPLC, consisted of a quaternary pump (Jasco PU-2089 Plus), a manual injector setup (50 µl loop) a degasser and a PDA (Spectra System UV 8000). The HPLC system was controlled by a Software ChromQuest 5.0. Chromatographic separation was carried out with a column Tracer $C_{18}$ (250 × 4.6 mm; 5 µm particle size) thermostatized at 30 ºC, at a flow rate of 0.8 ml/min. The mobile phase was a mixture of (A) ACN (B) Milli-Q water in a linear gradient starting with 20% (A) at 0 min, reaching 40% (A) at 8 min, 60% (A) at 27 min and 20% (A) at 30 min. Detection was performed at 240 nm. Identification of products was carried out by external standards, by comparison of retention time (allyl-isothiocyanate, phenethylITC, benzylITC, isopropylITC, ethylITC, goitrin, erucin, napin, sulforaphane and iberin).

### 2.3 Statistical analyses

Statgraphics Plus 5.1 statistical software (Statpoint Technologies, Inc., Warrenton, VA, USA) was used to perform a one-way analysis of variance (ANOVA), and multiple range tests were used to identify the differences between fresh samples and the samples subjected to the three treatments. The level of significance was set at $p<0.05$.

## 3. Results and discussion

The calibration lines were constructed by regressing obtained peak against the concentrations of the working solutions. The determination coefficient showed good linearity in a suitable range of concentrations depending on the expected results in the samples (Table 1). The detection limit was established according to ACS (1980). It was observed that goitrin has greater sensitivity with the lowest detection limit, while the sulforaphane presents the highest detection limit.

The accuracy and precision of the method were investigated by analysis of six replicates of steaming spiked turnip greens. As can be seen in Table 2, the results obtained demonstrated that the method was satisfactory.

Table 2. Precision (RSD %) and recovery (%)

Tabla 2. Precisión (RSD %) y recuperación (%)

| Analytes | RSD (%) | Recovery (%) |
|---|---|---|
| Goitrin | 2.75 | 82.8 |
| Iberin | 2.72 | 105 |
| Sulforaphane | 1.80 | 82.5 |
| AllylITC | 2.39 | 82.3 |
| Napin | 3.07 | 93.4 |
| Erucin | 2.69 | 88.4 |

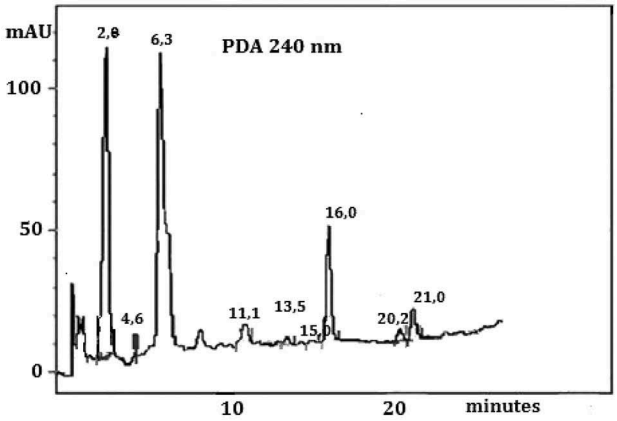


Figure 1. HPLC chromatogram (λ= 240 nm) with retention times of a fresh sample: goitrin (2.8), iberin (4.6), sulforaphane (6.3), ethylITC (11.1), allyilITC (13.5), isopropylITC (15.0), napin (16.0), benzylITC (20.2) and phenethylITC (21.0).

Figura 1. Cromatograma de HPLC (λ= 240 nm) con los tiempos de retención de muestra fresca: goitrina (2,8), iberina (4,6), sulforafano (6,3), etilITC (11,1), alilITC (13,5), isopropilITC (15,0), napina (16,0), bencilITC (20,2) and fenetilITC (21,0).

### 3.1 Identification and quantification of hydrolysis products in the samples

The moisture of the samples studied was about 90%. Because of its high moisture content, turnip greens are perishable foods, a feature that favors the growth of microorganisms, oxidation reactions and it affects the nutritional and organoleptic quality for short periods.

Eight compounds were identified in the four samples of turnip green fresh: allylITC, erucin, sulforaphane, iberin, napin, goitrin, benzylITC and phenethylITC. Hydrolysis products of aliphatic glucosinolates were the major compounds (71–97%) of the fresh sample (Figure 1) and treated with heat (75–95%). These data agree with the majority of studies (Volden, Wicklunf, Verkerk, & Dekker, 2008; Herr & Büchler, 2010; Bo, Na, Zhao, Yan, & Wang, 2011; Peñas, Frias, Martínez-Villaluenga, & Vidal-Valverde, 2011; Vicas, Teusdea, Carbunar, Socaci, & Socaciu, 2013; Korus et al. 2014). The average amounts of specific compounds found in turnip greens are presented in Figure 2.

In the studied samples of turnip greens, the predominant isothiocyanate is napin (26–33 mg/100 g dw). It is the 39–42% of the total isothiocyanates content. This agrees with other studies, (Cartea, Haro, Obregón, & Soengas, 2012) who found that gluconapine is the predominant glucosinolate. The gluconapine is related to the typical bitter flavour of the turnip greens; moreover it has nematicides, fungicides and insecticides. The goitrin is the second most abundant compound (17–23 mg/100 g dw). Hydrolysis of β-hydroxialkenyl glucosinolates, gives rise to β-hydroxyalkenyl isothiocyanates; these compounds, cyclize to oxazolidine-2-thiones (as goitrin). The phenetylITC also found in the samples of turnip greens, has been shown to inhibit the growth of lung and esophageal cancer in rat and mouse tumor models (Herr & Büchler, 2010) The rest of the isothiocyanates were found in lower amounts. EthylITC and isopropylITC were not detected.

Other researchers (Barbieri, Penice, Maggio, De Pascale, & Fogliano, 2008) have reported that 65–70% of all glucosinolate in *Brassica rapa* were the sum of gluconapin plus glucobrassicanapin. Sinigrin (SIN) and AllylITC are compounds found in vegetables of the *Brassica*, and recently, they have been used as a nutritional supplement (Okulicz, 2010; Bo et al. 2011).

### 3.2 Effect of treatments

Compared with fresh samples, all cooking methods were found to cause significant reduction in anthocyanin and total glucosinolates contents (Jones et al. 2010; Francisco et al. 2010; Korus et al. 2014; Xu et al. 2014). Heat treatments in Brassica affect the glucosinolate and the isothiocyanates.

Table 1. Concentration range, regression equation, coefficient of determination and detection limit of the analytes.

Tabla 1. Concentración, rango, ecuación de regresión, coeficiente de determinación y límite de detecciónde los analitos.

| | Range (mg/l) | Regression equation | Determination coefficient ($r^2$) | Detection limit (mg/l) |
|---|---|---|---|---|
| Goitrin | 0.235–60.3 | y = −16.3 + 49.5 x | 0.9998 | 0.059 |
| Iberin | 1.11–35.6 | y = 3.16 + 2.22 x | 0.9995 | 0.27 |
| Sulforaphane | 2.20–35.3 | y = −1.21 + 3.33 x | 0.9996 | 0.55 |
| AllylITC | 0.895–28.6 | y = −0.678 + 7.98 x | 0.9999 | 0.22 |
| Napin | 0.895–54.9 | y = −1.85 + 5.75 x | 0.9994 | 0.21 |
| Erucin | 1.19–38.2 | y = −5.09 + 9.39 x | 0.9992 | 0.26 |
| EthylITC | 0.777–99.0 | y = 4.78 + 13.6 x | 0.9999 | 0.19 |
| IsopropylITC | 0.740–94.8 | y = 1.22 + 10.6 x | 0.9999 | 0.18 |
| BenzilITC | 0.875–112 | y = -0.810 + 9.79 x | 0.9998 | 0.21 |
| PhenetylITC | 0.854–109 | y = 8.06 +7.26 x | 0.9996 | 0.21 |

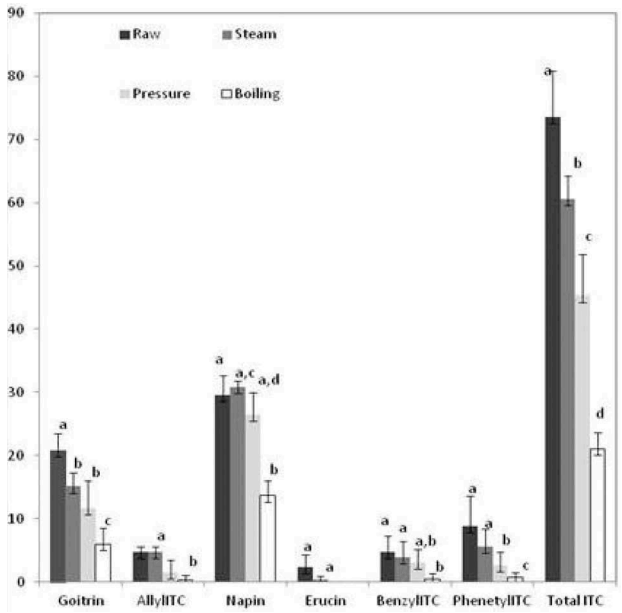


Figure 2. Mean values ± standard deviation of compounds obtained in fresh and processed turnip greens, expressed in mg/100g dry weight. For each compound, different letters among bars indicate significant differences at 0.05 % level.
[1]Values are mean of four samples, expressed in mg/100 g dry weight.
[2] For each compound, different letters among bars indicate significant differences ($p < 0.05$)

Figura 2. Valores medios ± desviación estandard de los compuestos obtenidos in hojas de greolo, frescas y procesadas, expresadas in mg/100g de muestra seca. Para cada compuesto, letras diferentes letters en las barras indican diferencias significativas a nivel del 0,05 %.

The effects of treatments are shown in Figure 2. Iberin, sulforaphane, and erucin are in small quantities in the fresh sample and only a small amount of erucin remains after steaming. Aliphatic glucosinolates were usually more stable than indole GLSs (Korus et al.2014)

Significant differences ($p<0.05$) in all compounds were found between steamed and boiled samples. These reductions ranged from 20–33% in pressure treatment except for napin and benzylITC. Higher losses were found in the boiled vegetables (45–60%). The content of hydrolysis products of aliphatic glucosinolates has shown a reduction from 5–12% in steamed, 18–23% in pressure-cooked and 37–45% in boiled. Goitrin suffered a greater reduction, according to Volden et al. (2008), in comparison with aliphatic glucosinolates. In general, the relative stabilities of individual glucosinolates may be a function of their respective chemical structures (Cieslik, Leszcynska, Filipiak-Florkiewicz, Sikora, & Pisulewski, 2007; Volden et al. 2008). Individual aliphatic glucosinolates glucoiberin, glucoraphanin, and glucoalyssin are more susceptible than sinigrin and gluconapin (Cartea & Velasco, 2008).

In accordance with the data obtained, steaming better preserves all glucosinolates (Figure 2). Several studies have shown that microwaving and boiling are the cooking methods that cause the largest losses (Cieslik et al. 2007; Sarvan, Verkerk, & Dekker, 2012; Korus et al. 2014; Xu et al. 2014). In comparison, steaming causes less loss of glucosinolates in broccoli, brussels sprouts, cauliflower and cabbage (Cieslik et al. 2007; Gao-Feng et al. 2009; Jones et al. 2010; Korus et al. 2014). These results agree with studies about different cooking conditions on broccoli samples (Bongomi, Verkek, Steenbekkers, Dekker, & Stieger, 2014). Steaming treatment showed an increase (+17%) of the amount of total glucosinolates and boiling showed a decrease (–40, –50%) in the amount of total glucosinolates.

## 4. Conclusions

This study indicates that turnip greens can be considered a good source of bioactive compound, namely, glucosinolates and their hydrolysis products, but the cooking treatments prior to consumption produce a significant decrease in their content. These processes require optimization to prevent their loss and provide a potential benefit to human health. The best cooking process of the three studied, is steaming that produces lesser reductions, followed by pressure-cooking, while boiling produces a 60% loss of the glucosinolate content.

## Disclosure statement

No potential conflict of interest was reported by the authors.

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